

Supplemental Information

Crystal Structure of Chiral γ PNA with Complementary DNA Strand—Insights into the Stability and Specificity of Recognition and Conformational Preorganization

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Table of Contents

	Page
Figure S1. CD spectra of single-stranded PNA and γ PNA, and the corresponding PNA-DNA and γ PNA-DNA hybrid duplexes	2
Figure S2. Crystal packing of γ PNA-DNA double helices	3
Figure S3. View of γ PNA-DNA double helices coordinating with Mg ²⁺ and Zn ²⁺	4
Figure S4. CD spectra and UV-melting profiles of γ PNA-DNA duplex in different buffer conditions	5
Figure S5. ¹ H-NMR spectra of (T) Gly- γ PNA, ^L Ala- γ PNA, ^L Val- γ PNA and ^L Ile- γ PNA monomers	6
Figure S6. ¹ H-NMR spectra of (T) ^L Ile- γ PNA and the various substructures	7
Figure S7. ¹ H-NMR spectra of ^L Ile- γ PNA without the nucleobase and linker	8
Table S1. Helical parameters of the DNA- γ PNA duplex (AC/BD)	9
Table S2. DNA torsion angles (AC/BD)	10
Table S3. Pseudorotation angles of the sugar ring of the AC duplex	11
Table S4. Pseudorotation angles of the sugar ring of the BD duplex	12
Table S5. γ PNA torsion angles (AC/BD)	13
Table S6. ¹ H-NMR assignments of (T) Gly- γ PNA, ^L Ala- γ PNA, ^L Val- γ PNA and ^L Ile- γ PNA monomers	14
Table S7. ¹ H-NMR assignments of (T) ^L Ile- γ PNA and the various substructures	15

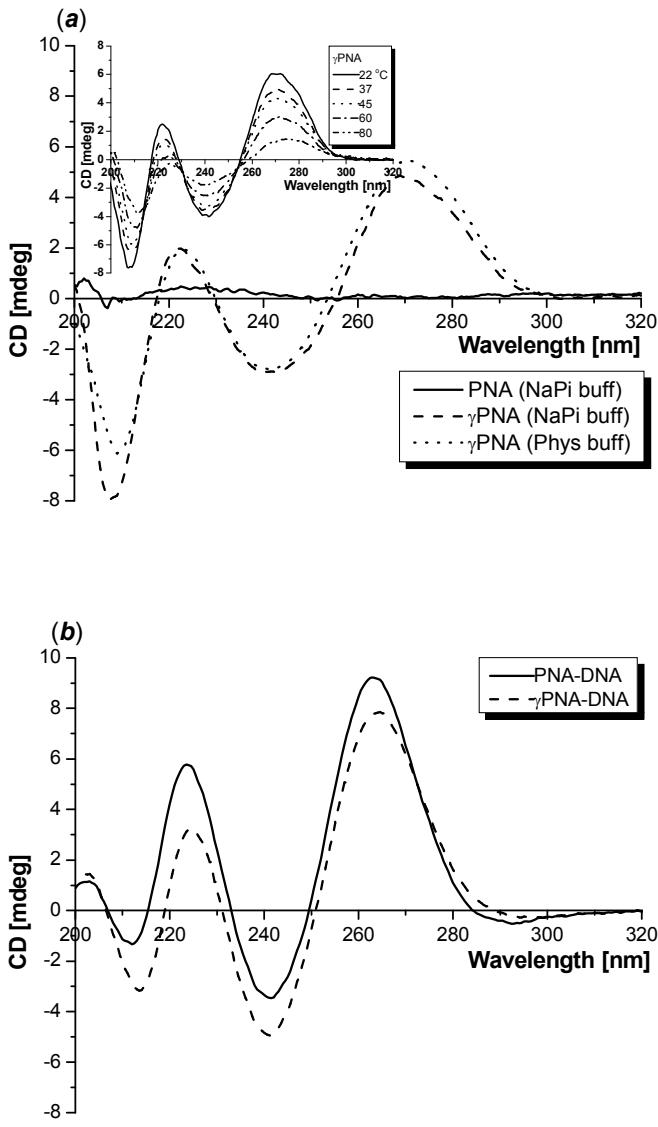


Figure S1. (a) CD spectra of single-stranded PNA and γ PNA oligomers, and (b) hybridized PNA-DNA and γ PNA-DNA duplexes. *Inset in (a)*: CD spectra of a single-stranded γ PNA recorded at different temperatures. The samples were prepared in NaPi (10 mM sodium phosphate, pH 7.4) or in Phys buffer (2 mM MgCl₂, 150 mM KCl, pH 7.4) as indicated at 5 μ M strand concentration each. Notice that the total strand concentration of the hybrid duplex in (b) is twice as that of the individual γ PNA strand in (a)—hence the doubling in the amplitude of the CD signals. The sequence of PNA oligomer is the same as that of γ PNA shown in Figure 1 but without the γ -backbone modifications.

- The CD profile of single-stranded γ PNA, with maxima at 220 and 270 nm and minima at 210 and 240 nm, is characteristic of a right-handed helix.¹
- The ability of single-stranded γ PNA to adopt a right-handed helix is independent of ionic strength, as indicated by the similarities in the CD profiles of samples prepared in NaPi and in Phys buffer.
- The CD profiles of PNA-DNA and γ PNA-DNA duplexes, with a strong positive peak at \sim 260 nm and a steep negative ellipticity at \sim 210 nm, suggest an A-DNA type base-stacking.²

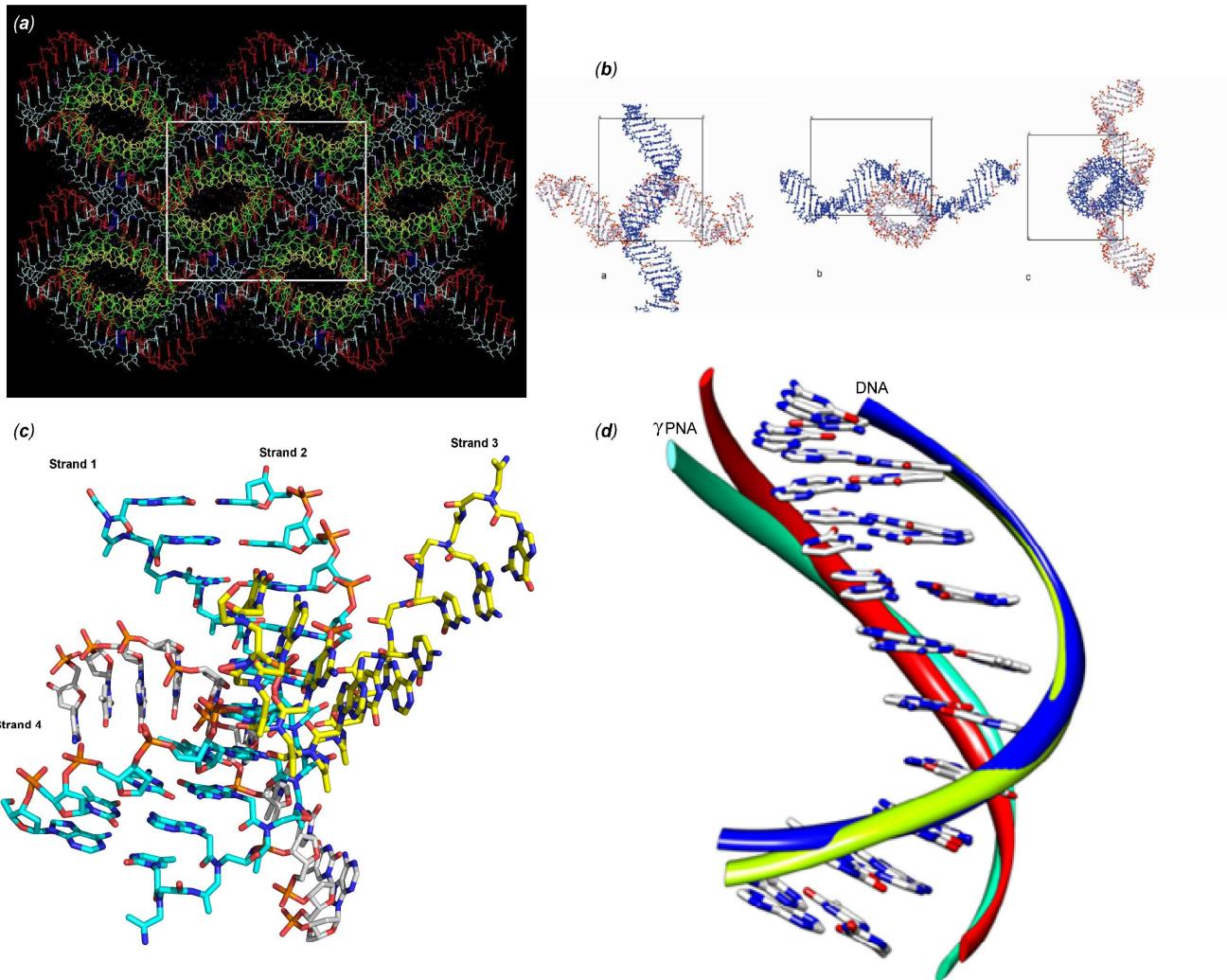


Figure S2. (a) Crystal packing of γ PNA-DNA double helices is positioned at 90° and propagated along the b and c crystallographic axis. The three-dimensional packing produced large cavities that accommodate $\sim 63\%$ of the solvent molecules present in the structure. (b) View along the a-axis, b-axis and c-axis. (c) View of the γ PNA-DNA double helices in the asymmetric unit. (d) Superposition of the two γ PNA-DNA double helices.

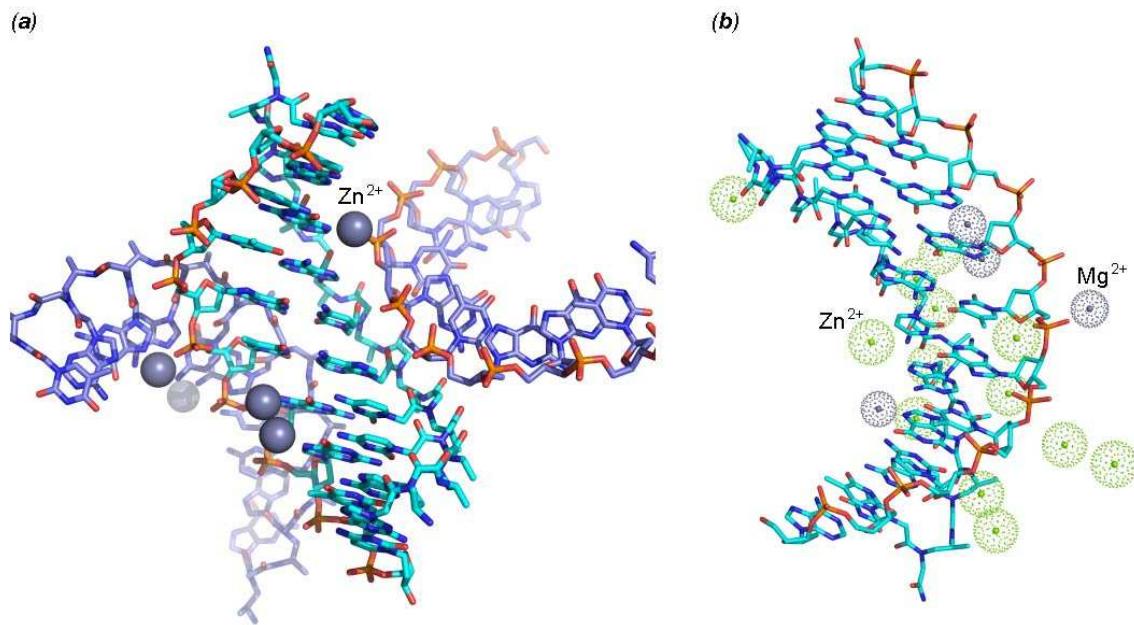


Figure S3. (a) View of the γ PNA-DNA double helices in the asymmetric unit with Zn^{2+} atoms coordinating with the phosphate groups in the DNA backbone and nucleobases of γ PNA (enhancing lattice interactions), and (b) a more detailed few of the interactions between Mg^{2+} and Zn^{2+} with DNA and γ PNA strands.

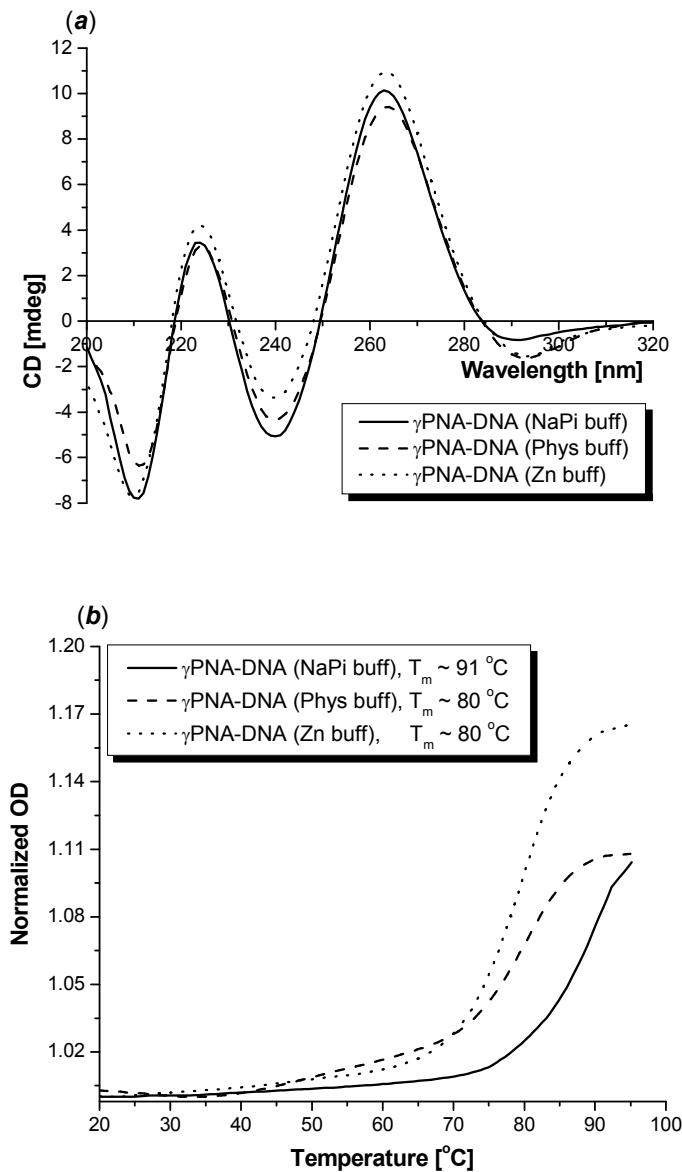


Figure S4. (a) CD spectra of the γ PNA-DNA hybrid duplex prepared in NaPi buffer (10 mM sodium phosphate, pH 7.4), physiological buffer (2 mM $MgCl_2$, 150 mM KCl, pH 7.4), and Zn buffer (5 mM $ZnSO_4$, used to derivatize the crystals for anomalous phasing); and (b) UV-melting profile of the respective sample. Notice that the melting transitions (T_m s) of the duplex in Phys and in Zn buffer are ~ 10 °C lower than that in NaPi buffer (relatively low ionic strength).

- The similarities in the CD profiles of the γ PNA-DNA duplex in the three buffer systems indicate that neither Zn^{2+} nor Mg^{2+} has any effect on the conformation of the duplex.
- The difference in the T_m s of the PNA-DNA duplex noted above has been documented.³ It can be explained in term of counterion release upon hybridization in contrast to counterion association observed with formation of DNA duplex.

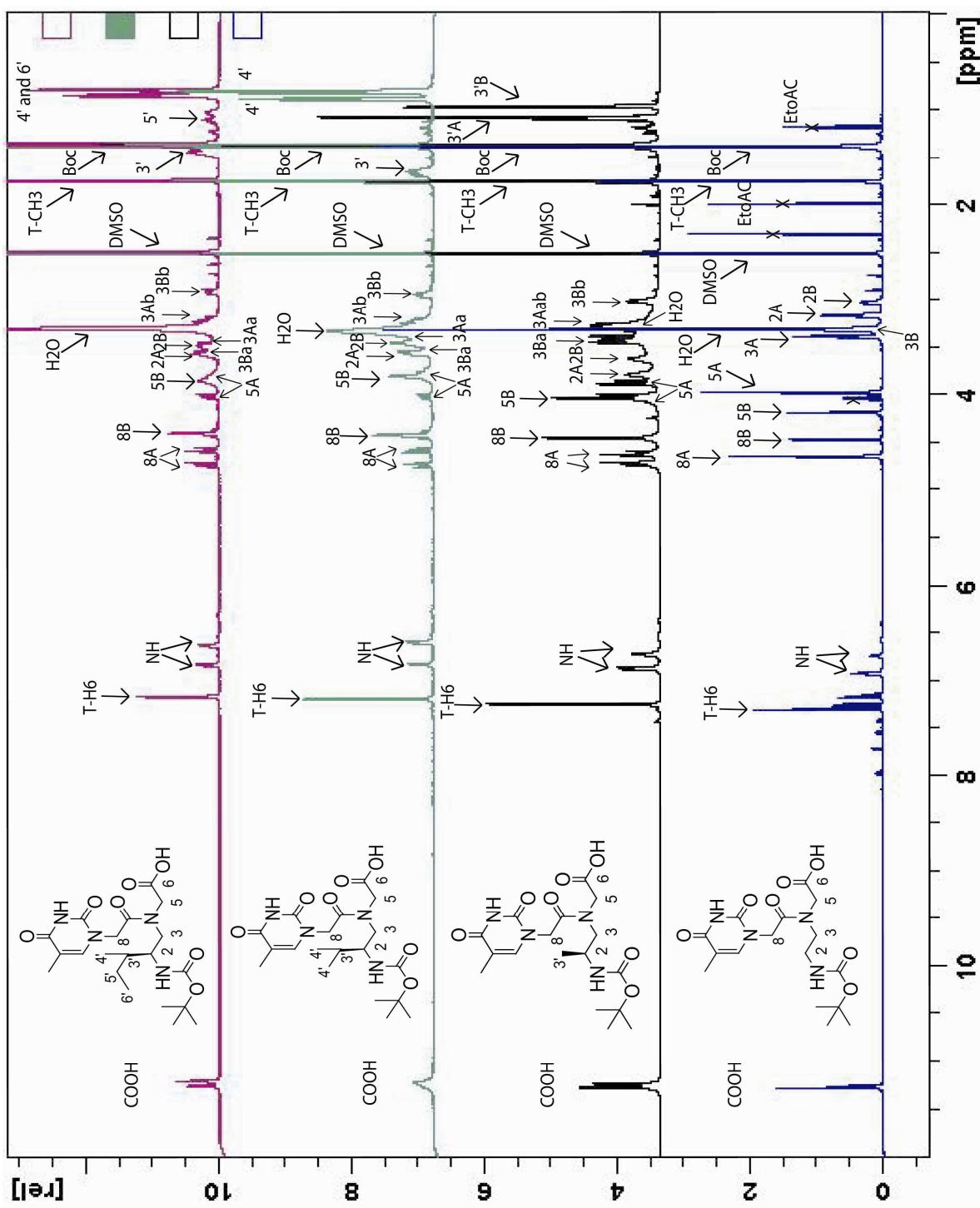


Figure S5. ¹H-NMR spectra of (^LT) Gly- γ PNA, ^LAla- γ PNA, ^LVal- γ PNA and ^LIle- γ PNA monomers (500.13 MHz – DMSO-*d*₆)

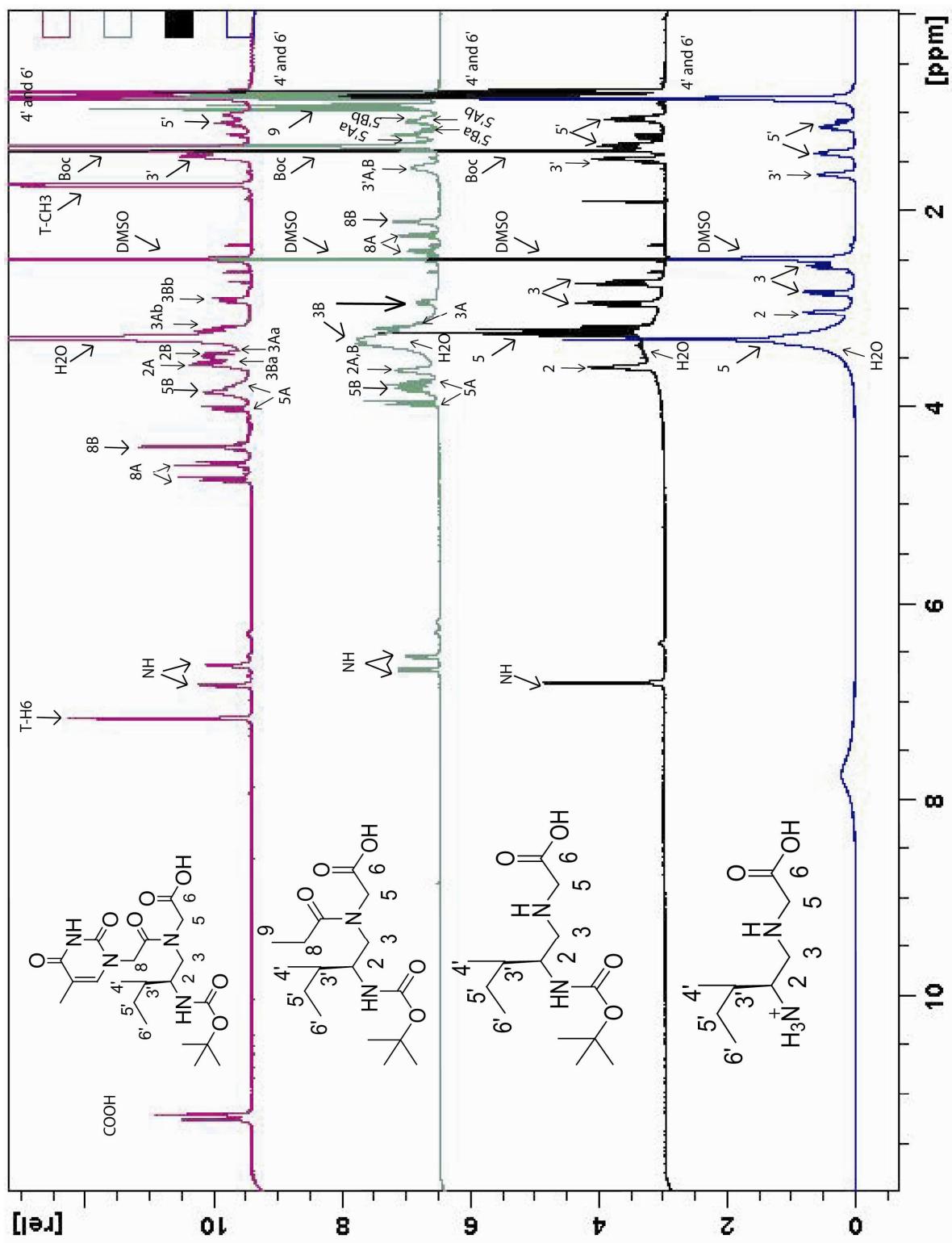


Figure S6. ¹H-NMR spectra of (T)^LIle-g-PNA and the various substructures (500.13 MHz – DMSO-*d*₆)

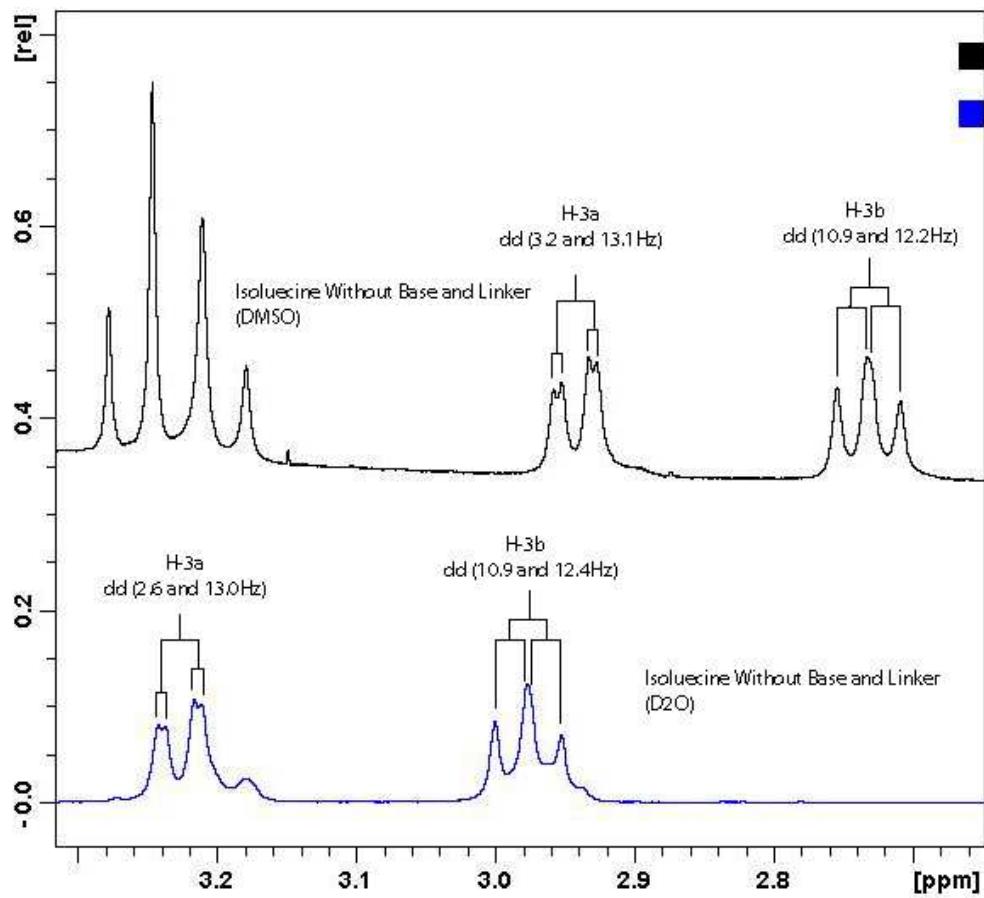


Figure S7. ¹H-NMR showing the region of a preferred conformational structure. The submonomer unit of isoleucine without base and linker shows a preferred conformational structure in the NMR regions 2.65-3.35 ppm in both DMSO-*d*₆ (top) and D₂O (bottom). Similar to the isoleucine thymine monomers, the ³*J*-coupling constants are ~12-13 and 11 Hz for the A component and ~13 and 3 Hz for the B component of the AB system indicating the presence of a preferred structure.

Table S1. Helical parameters of the γ PNA-DNA duplexes (AC/BD)*

Global base pair [†]	x-displacement (Å)	Inclination °	Local inter-base pair	Twist °	Roll °	Slide (Å)
<i>dA1-Tp10</i>	-5.35/-6.09	2.77/-1.16	<i>dA1-Tp10/ dT2-Ap9</i>	23.24/22.54	1.76/-0.62	-2.19/-2.89
<i>dT2-Ap9</i>	-5.40/-6.02	4.53/2.04	<i>dT2-Ap9/ dC3-Gp8</i>	26.54/26.20	1.21/5.65	-1.90/-2.31
<i>dC3-Gp8</i>	-5.48/-6.13	4.12/3.28	<i>dC3-Gp8/ dT4-Ap7</i>	25.58/15.97	4.89/7.76	-2.36/-1.86
<i>dT4-Ap7</i>	-5.63/-6.54	2.27/6.72	<i>dT4-Ap7/ dG5-Cp6</i>	24.36/23.25	3.62/8.75	-2.12/-2.34
<i>dG5-Cp6</i>	-5.58/-6.28	-1.48/5.71	<i>dG5-Cp6/ dT6-Ap5</i>	21.28/27.13	-1.84/1.24	-2.86/-2.67
<i>dT6-Ap5</i>	-5.57/-6.29	-1.83/5.73	<i>dT6-Ap5/ dG7-Cp4</i>	24.74/23.11	3.34/6.76	-2.21/-1.89
<i>dG7-Cp4</i>	-5.69 /-6.40	-1.52/5.05	<i>dG7-Cp4/ dG8-Cp3</i>	20.72/20.09	2.34/6.26	-2.72/-2.82
<i>dG8-Cp3</i>	-5.58/-6.14	-2.48/5.11	<i>dG8-Cp3/ dT9-Ap2</i>	24.15/25.25	-3.53/-1.38	-2.39/-2.25
<i>dT9-Ap2</i>	-5.65/-6.25	-0.19/5.36	<i>dT9-Ap2/ dC10-Gp1</i>	24.63/24.51	5.65/2.17	-2.33/-2.30
<i>dC10-Gp1</i>	-5.62/-6.35	0.74/6.26				

*AC/BD refers to the two duplexes in the asymmetric unit

[†]*d* refers to DNA strand, 5'-end corresponds to position 1; *p* refers to γ PNA strand, N-terminus corresponds to position 1.

Table S2. DNA torsion angles* (AC/BD), [°]

Monomer	α	β	γ	δ	ϵ	ζ	χ
<i>dA1</i>	-----	-170/169	90/102	26/4	-158/-147	-25/-60	-146/170
<i>dT2</i>	-119/-71	-145/165	118/74	41/63	-146/-144	-78/-76	-129/-160
<i>dC3</i>	140/-51	180/168	-174/94	85/12	-153/-142	-77/-74	-163/-158
<i>dT4</i>	-70/-60	169/-120	56/55	79/78	-156/-168	-72/-79	-158/-146
<i>dG5</i>	-76/157	-176/-175	66/-159	81/18	-166/-132	-67/-68	-169/179
<i>dT6</i>	-75/-67	170/170	65/48	82/84	-154/-153	-66/-73	-161/-156
<i>dG7</i>	-74/-83	170/108	53/65	83/85	-165/-161	-64/-96	-164/-159
<i>dG8</i>	-90/36	165/175	82/113	82/-82	-157/-119	-89/-35	-175/-170
<i>dT9</i>	-38/-73	160/-176	43/75	89/60/-157/-58	-155/-156
<i>dC10</i>/-52	33/44	93/101	-157/-145
Average	-50/-69	180/174	43/51	74/42	-157/-147	-67/-69	-158/-160
A-DNA ⁴	-50	172	41.1	79	-146	-78	-154
B-DNA ⁴	-46	-147	36	157	155	-96	-98
α PNA-DNA ⁵	-35	162	65.6	108.2	-159.0	-80.7	-116.1
PNA-DNA ⁶	-71	-165	49	110	-171	-75	-134
PNA ₂ -DNA ⁴	-70	173	61	77	-161	-69	-167

* **α :** O3'(i-1)-P-O5'-C5'; **β :** P-O5'-C5'-C4'; **γ :** O5'-C5'-C4'-C3'; **δ :** C5'-C4'-C3'-O3'; **ϵ :** C4'-C3'-O3'-P O3'-P(i+1); **ζ :** C3'-O3'-P(i+1)-O5'(i+1); **χ :** O4'-C1'-N1-C2 (py), O4'-C1'-N9-C4 (pu).

AC/BD refers to the two duplexes in the asymmetric unit. For calculating the average, negative β were taken as $(360^\circ - |\beta|)$.

Table S3. Pseudorotation angles of the sugar rings of the AC duplex, [°]

Base	v0	v1	v2	v3	v4	tm	P	Puckering
<i>dA1</i>	-16.4	31.9	-33.9	24.7	-5.2	34.4	169.9	C2'-endo
<i>dT2</i>	-28.5	49.6	-48.9	33.7	-1.8	50.9	164.2	C2'-endo
<i>dC3</i>	12.6	-33.1	38.8	-33.0	11.6	38.8	359.5	C2'-exo
<i>dT4</i>	0.2	-23.9	36.4	-37.5	22.4	38.3	17.7	C3'-endo
<i>dG5</i>	1.0	-25.2	37.4	-38.1	22.5	39.1	16.7	C3'-endo
<i>dT6</i>	-3.4	-20.9	35.4	-39.1	25.4	38.6	23.4	C3'-endo
<i>dG7</i>	-2.1	-24.0	38.0	-39.9	26.0	40.6	20.6	C3'-endo
<i>dG8</i>	-8.9	-16.7	33.5	-40.1	29.8	39.1	31.1	C3'-endo
<i>dT9</i>	-8.7	-12.7	28.1	-33.5	25.8	33.3	32.6	C3'-endo
<i>dC10</i>	4.6	-23.1	30.5	-29.2	14.5	30.9	9.7	C3'-endo

v0: C4'-O4'-C1'-C2', v1: O4'-C1'-C2'-C3', v2: C1'-C2'-C3'-C4', v3: C2'-C3'-C4'-O4', v4: C3'-C4'-O4'-C1', tm and P: amplitude and phase angle of pseudorotation of the sugar ring, respectively. Sugar parameters were analyzed with 3DNA.⁷

Table S4. Pseudorotation angles of the sugar rings of the BD duplex, [°]

Base	v0	v1	v2	v3	v4	tm	P	Puckering
<i>dA1</i>	52.9	-51.2	27.8	3.3	-34.2	53.8	301.1	C1'-endo
<i>dT2</i>	-16.2	8.1	2.5	-12.0	16.8	17.5	81.8	O4'-endo
<i>dC3</i>	41.2	-41.9	21.8	3.0	-28.3	43.1	300.4	C1'-endo
<i>dT4</i>	-4.4	-18.8	33.1	-37.1	25.1	36.6	25.1	C3'-endo
<i>dG5</i>	41.8	-37.5	16.9	7.6	-30.8	41.8	293.9	C1'-endo
<i>dT6</i>	0.9	-24.8	36.7	-37.7	22.1	38.3	16.8	C3'-endo
<i>dG7</i>	-2.8	-20.4	33.2	-36.4	23.7	36.0	22.6	C3'-endo
<i>dG8</i>	13.0	14.2	-32.9	41.4	-33.5	40.7	216.1	C4'-endo
<i>dT9</i>	-9.9	2.2	5.8	-11.6	12.7	13.2	63.8	C4'-exo
<i>dC10</i>	-9.5	-5.6	17.1	-23.8	20.1	23.2	42.3	C4'-exo

v0: C4'-O4'-C1'-C2', v1: O4'-C1'-C2'-C3', v2: C1'-C2'-C3'-C4', v3: C2'-C3'-C4'-O4', v4: C3'-C4'-O4'-C1', tm and P: amplitude and phase angle of pseudorotation of the sugar ring, respectively. Sugar parameters were analyzed with 3DNA.⁷

Table S5. γ PNA torsion angles (AC/BD), [$^\circ$]

Monomer	α	β	γ	δ	ϵ	ω	χ_1	χ_2	χ_3
Tp10	N/A	-71/165	45/36	124/113	-78/-156	-151/-72	15/14	-168/-172	85/84
Ap9	-97/-104	75/75	52/50	141/130	-110/-143	-136/-102	-14/16	-161/178	93/76
Gp8	-100/-93	58/53	66/62	123/120	-86/-146	-136/-68	12/23	-166/178	-1/17
Ap7	-101/-91	63/55	62/60	125/120	-103/-157	-155/-95	19/16	-176/178	75/87
Cp6	-101/-109	68/78	70/68	103/71	-138/174	-147/-129	9/14	-170/176	81/86
Ap5	-108/-131	66/69	60/55	117/115	-121/-151	-152/-141	14/15	-174/-176	80/89
Cp4	-103/-92	82/64	42/66	171/116	-134/-160	-174/-172	3/-5	-174/-173	81/105
Cp3	-71/-107	51/87	58/58	77/138	-68/-143	179/-107	28/23	169/-168	77/81
Ap2	-115/-117	77/81	60/58	123/125	-120/-152	-155/-114	4/-3	-176/-170	86/-78
Gp1	-82/-94	49/63	94/82	81/95	-32/-25	N/A	-7/-3	-164/-170	98/87

γ PNA torsion angle definitions: $\alpha=C6'-N1'-C2'-C3'$; $\beta=N1'-C2'-C3'-N4'$; $\gamma=C2'-C3'-N4'-C5'$; $\delta=C3'-N4'-C5'-C6'$; $\epsilon=N4'-C5'-C6'-N1'$; $\omega=C5'-C6'-N1'-C2'$; $\chi_1=C3'-N4'-C7'-C8'$; $\chi_2=N4'-C7'-C8'-N1(\text{py})/\text{N9}(\text{pu})$; $\chi_3=C7'-C8'-N1(\text{py})/\text{N9}(\text{pu})-\text{C2}(\text{py})/\text{C4}(\text{pu})$.

Table S6. ^1H -NMR-assignments of (T) Gly- γ PNA, $^L\text{Ala-}\gamma\text{PNA}$, $^L\text{Val-}\gamma\text{PNA}$ and $^L\text{Ile-}\gamma\text{PNA}$ monomers (500.13 MHz – DMSO- d_6)^a

H	Unmodified		Alanine		Valine		Isoleucine	
Rotamer	A	B	A	B	A	B	A	B
NH	6.92 t J=5.9Hz	6.73 t J=5.9Hz	6.87 d J=8.4Hz	6.72 d J=7.8Hz	6.83 d J=9.9Hz	6.59 d J=9.0Hz	6.83 d J=9.7Hz	6.63 d J=9.2Hz
2	3.16 q J=6.1Hz	3.02 q J=6.4Hz	3.78 m	3.62 m	3.55 m	3.44 m	3.58 m	3.50 m
3a	3.38 t J=6.7Hz	3.3 under H_2O	3.30 dd J=5.9 and 15.1 Hz	3.44 dd J=6.5 and 13.6Hz	3.47 dd J=4.6 and 14.5Hz	3.56 dd J=4.6 and 13.6Hz	3.46 dd J=2.9 and 15.0 Hz	3.55 dd J=5.1 and 13.9Hz
3b	NA	NA	3.24 dd J=8.4 and 14.6Hz	3.00 dd J=6.8 and 13.7Hz	3.22 dd J=10.2 and 14.6Hz	2.93 dd J=8.8 and 13.0Hz	3.20 dd J=11.3 and 14.9Hz	2.90 dd J=9.3 and 12.5Hz
5a	3.96 s	4.18 s	4.00 d J=17.3Hz	4.03 s	4.01 d J=17.4Hz	3.80 bq J=17.4Hz	4.01 d J=16.9Hz	3.84 bs
5b	NA	NA	3.86 d J=17.3Hz	NA	3.73 d J=17.4Hz	NA	3.78 d J=16.9Hz	NA
8a	4.63 s	4.46 s	4.73 d J=16.9Hz	4.45 s	4.74(a) d J=16.3Hz	4.44 d J=16.3Hz	4.73 d J=16.6Hz	4.42 d J=17.0Hz
8b	NA	NA	4.60 d J=16.9Hz	NA	4.58 d J=16.3Hz	4.39 d J=16.3Hz	4.57 d J=16.6Hz	4.38 d J=17.0Hz
3'	NA	NA	1.07 d J=6.9Hz	0.95 d J=6.8Hz	1.65m	1.65m	1.44 m	1.38 m
4'	NA	NA	NA	NA	0.88 dd J=4.9 and 6.2Hz, 0.80 dd J=6.7 and 11.1Hz	0.88 dd J=4.9 and 6.2Hz, 0.80 dd J=6.7 and 11.1Hz	0.85 d	0.78 d
5'	NA	NA	NA	NA	NA	NA	1.10 m	1.02 m
6'	NA	NA	NA	NA	NA	NA	0.83 t	0.79 t
Boc	1.38 s	1.37 s	1.36 s	1.37 s	1.38 s	1.36 s	1.36 s	1.34 s
T-H6	7.30 s	7.30 s	7.25 s	7.24 s	7.19 s	7.19 s	7.16 s	7.16 s
T-CH₃	1.75 s	1.75 s	1.75 s	1.75 s	1.75 s	1.75 s	1.73 s	1.74 s
COOH	11.27 s	11.25 s	11.27 s	11.23 s	11.24 s	11.23 s	11.25 s	11.21 s

^abq = broad quartet, bs = broad singlet, NA = Not Assigned

Table S7. ^1H NMR assignments of (T) $^{\text{L}}\text{Ile-}\gamma\text{PNA}$ and the various substructures (500.13 MHz – DMSO- d_6)

H	Ile (Base, Linker, and Boc Removed)	Ile (Base and Linker Removed)	Ile (Base Removed)		Isoleucine	
Rotamer			A	B	A	B
NH	7.74 b	6.81 d	6.67 d $J=9.2\text{Hz}$	6.54 d $J=9.5\text{Hz}$	6.83 d $J=9.7\text{Hz}$	6.63 d $J=9.2\text{Hz}$
2	3.03 m	3.60 m	3.62(A,B) m		3.58 m	3.50 m
3a	2.83 dd $J=2.5$ and 13.0Hz	2.94 dd $J=3.2$ and 13.1Hz	3.19 m under H_2O	3.37 dd $J=5.7$ and 13.9Hz	3.46 dd $J=2.9$ and 15.0 Hz	3.55 dd $J=5.1$ and 13.9Hz
3b	2.56 dd $J=9.8$ and 12.6Hz	2.73 dd $J=10.9$ and 12.2Hz		2.93 dd $J=8.5$ and 13.3Hz	3.20 dd $J=11.3$ and 14.9Hz	2.90 dd $J=9.3$ and 12.5Hz
5a	3.31 Under H_2O	3.23 q $J=15.8\text{Hz}$	3.85 d $J=17.7\text{Hz}$	3.81 d $J=18.3\text{Hz}$	4.01 d $J=16.9\text{Hz}$	3.84 bs
5b	NA	NA	NA	NA	3.78 d $J=16.9\text{Hz}$	NA
8a	NA	NA	2.40 m and 2.24 m	2.10 m	4.73 d $J=16.6\text{Hz}$	4.42 d $J=17.0\text{Hz}$
8b	NA	NA	NA	NA	4.57 d $J=16.6\text{Hz}$	4.38 d $J=17.0\text{Hz}$
9	NA	NA	0.96 t $J=7.3\text{Hz}$	0.91 t $J=7.3\text{Hz}$	NA	NA
3'	1.63m	1.47m	1.56(A,B)m		1.44 m	1.38 m
4'	0.85m	0.77d $J=6.9\text{Hz}$	0.82m		0.85 d	0.78 d
5'	1.41	1.33m and 1.05m	1.29(a)m and 1.10(b)m	1.20(a) m and 1.05(b) m	1.10 m	1.02 m
6'	0.85m	0.82m $J=7.3\text{Hz}$	0.82m		0.83 t	0.79 t
Boc	NA	1.38 s	1.34 s	1.32 s	1.36 s	1.34 s
T-H6	NA	NA	NA	NA	7.16 s	7.16 s
T-CH₃	NA	NA	NA	NA	1.73 s	1.74 s
COOH	NA	NA	NA	NA	11.25 s	11.21 s

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